Evaluation of the Efficacy and Safety of Rifaximin in Combination with N-acetylcysteine (NAC) in Adult Patients with Irritable Bowel Syndrome with Diarrhea

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Summary of Changes from Previous Version:

Affected Section(s)	Summary of Revisions Made	Rationale
6.2.1	Acceptability of 90% study drug compliance was added	Drug adherence is crucial to clinical trials. However, subjects are being asked to take 6 capsules daily. It is implausible to expect 100% compliance

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STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable United States (US) Code of Federal Regulations (CFR). The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the Investigational New Drug (IND) sponsor, funding agency and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title: Eva

Evaluation of the Efficacy and Safety of Rifaximin in combination with N-acetylcysteine (NAC) in Adult Patients with Irritable Bowel Syndrome with Diarrhea (IBS-D)

Study Description:

Rifaximin is a minimally absorbed antibiotic that specifically targets the gastrointestinal tract and is FDA-approved for the treatment of IBS-D. Although treatment with rifaximin is extremely effective, symptom recurrence is common. The mucus layer in the small intestine is viscous (1, 2), which can render the microbes in the mucus layer inaccessible. This inaccessibility may protect certain bacterial species from the effects of rifaximin, facilitating their regrowth and the recurrence of symptoms.

The reducing agent dithiothreitol (DTT) can reduce the disulfide bonds linking mucin subunits in mucus (3), and we have recently used it to reduce viscosity and improve bacterial recovery from small intestinal samples (4). This led us to hypothesize that using rifaximin in combination with a mucolytic agent would make mucosal bacteria more accessible and thus improve the efficacy of rifaximin in treating IBS symptoms. DTT is not approved for use in humans, but the mucolytic agent N-acetylcysteine (NAC) has been used in the management of chronic obstructive pulmonary disease (COPD) (5) and other conditions (6). In this study, we propose to test our hypothesis by comparing the efficacies of different doses of rifaximin in combination with NAC vs. rifaximin alone in decreasing clinical symptoms in subjects with IBS-D.

Objectives:

Primary Objective: To assess the efficacy of combined Rifaximin and NAC therapy vs. Rifaximin alone in decreasing clinical symptoms in subjects with IBS-D.

Secondary Objectives (Exploratory):

- To determine the effects of rifaximin in combination with NAC on the gut microbiome
- To determine whether artificial intelligence characterization of Bristol stool scores (via the Dieta App) for stool photos can be used to normalize stool scores

Endpoints:

Primary Endpoints:

- Improvement in stool form and reduction in stool frequency from baseline, as determined from stool diary data comparing rifaximin alone vs rifaximin and NAC
- 2. Decrease in severity of abdominal pain from baseline, as determined from weekly average visual analog scale (VAS) scores, relative to rifaximin alone

Secondary Endpoints:

- 1. Improvement in urgency from baseline, as determined from weekly average VAS scores, relative to rifaximin alone
- 2. Improvement in bloating from baseline, as determined from weekly average VAS scores, relative to rifaximin alone
- 3. Reduction of H₂ on lactulose hydrogen breath test (LHBT) from baseline, relative to rifaximin alone

Exploratory Endpoints:

- 1) Changes in the stool microbiome from baseline, as determined by 16S rRNA gene sequencing
- 2) Normalization of stool scores from baseline

Study Population:

Adult patients (age 18-75) with IBS-D (diagnosed based on Rome IV criteria) will be assessed for eligibility to participate in this study.

Phase:

Phase I

Description of Sites/Facilities Enrolling Participants: Description of Study

Intervention:

Cedars-Sinai Medical Center

The proposed study is a prospective proof-of-concept double-blind (to dose of NAC) clinical trial to determine the efficacy of combined rifaximin and NAC therapy vs. rifaximin alone in decreasing clinical symptoms in subjects with IBS-D.

The study is a single-site, open-label trial which will consist of 3 phases:

- 1) Run-In Phase: Eligible subjects with IBS-D who consent to participate will be recruited. After provision of informed written consent, these subjects will undergo screening and baseline testing as described in the Schedule of Activities and will complete a daily stool diary for 14 days as well as weekly symptom questionnaires.
- 2) <u>Treatment Phase:</u> Subjects (n=45) will be randomized in (blocks of 3) to one of 3 study arms and will receive either:
 - a. Rifaximin 550 mg by mouth three times daily for 14 days (N=15)

OR

b. Rifaximin 200 mg plus placebo by mouth three times daily for 14 days (N=15).

OR

c. Rifaximin 200 mg plus N-acetylcysteine 600mg by mouth three times daily for 14 days (N=15)

Subjects will complete a daily stool diary for 14 days of treatment as well as weekly symptom questionnaires.

3) <u>Follow-Up Phase:</u> Upon completion of treatment, subjects will undergo repeat testing as at baseline. Subjects will then complete a daily stool diary for 28 days as well as weekly symptom questionnaires.

At the end of this phase, subject will complete an end-of-study visit with additional repeat testing as described in the Schedule of Activities.

Study Duration: The estimated study duration is 12 months.

Participant Duration: The participant duration will be approximately 8 weeks.

1.2 SCHEMA

Run-In Phase: Day -17 to Day -1

- Visit 1: Day -17 to -15
 - Baseline testing including medical history, symptom questionnaire, blood sample, breath test (details in Schedule of activities (SOA))
 - Pregnancy test (women of childbearing potential only)
 - Obtain stool collection kit (optional)
- At Home: Day -14 to Day -1
 - · Daily stool diary
 - Weekly symptom questionnaires
 - Phone Visit Day -7

Treatment Phase: Day 0 to Day 13

- Visit 2: Day 0 (RANDOMIZATION VISIT)
 - · Return optional stool sample
 - Return stool diaries and symptom questionnaires
 - Pregnancy test (women of childbearing potential only)
 - Randomize to receive either:
 - Rifaximin 550 mg 3x daily

OR

• Rifaximin 200 mg 3x daily + placebo

OR

- Rifaximin 200 mg 3x daily + NAC 600 mg 3x daily
- Obtain stool sample kit (optional)
- At Home: Day 0 to Day 13
 - · Daily stool diary
 - Weekly symptom questionnaires
 - Phone Visit Day 7

Follow-Up Phase: Day 14 to Day 45

- Visit 3: Day 14
 - Return optional stool sample, stool diaries and symptom questionnaires
 - · Return unused medications
 - Follow-up testing including physical exam, breath test, blood sample (details in SOA)
 - Pregnancy test (women of childbearing potential only)
 - Obtain stool collection kit (optional)
- At Home: Day 14 to Day 41
 - Daily stool diary
 - Weekly symptom questionnaires (phone visit scheduled)
 - Phone Visit Day 21
 - Phone Visit Day 28
 - Phone Visit Day 35
- Visit 4: Day 42 to 45
 - Submit stool diary questionnaires. Return stool sample (optional)
 - Blood sample, breath test

1.3 SCHEDULE OF ACTIVITIES (SOA)

Procedures	Visit 1 (Screening) Day -15 to -17	Run-In Days -14 to -1	Phone Visit – Day -7	Visit 2 (Randomization) Day 0	Treatment Days 0 to 13	Phone Visit – Day 7	Visit 3 Day 14 to 18	Follow-Up Days 14 to 41	Phone Visit – Day 21	Phone Visit – Day 28	Phone Visit – Day 35	Visit 4 Day 42 to 46
Informed consent	Х											
Medical history	Х											
Concomitant medication review	Х						Х					Х
Physical exam (including height and weight)	Х						Х					Х
Vital signs	Х						Χ					Х
Assessment for hematochezia, including FOBT	Х											
Urine pregnancy test (if required*)	Х			Х			Χ					
Serum pregnancy test (if required*)	Х			Х			Χ					
Blood draw (CBC, CMP, ESR, cytokines)	Х						Χ					Х
Lactulose breath test	Х						Χ					Х
Clinical Symptom Questionnaires (VAS)	Х						Χ					Х
Provide stool collection kit (optional)	Х			Х			Χ					
Return stool sample (exploratory for microbiome)				Х			Χ					Х
Adverse event review and evaluation	Х		Х	Х		Χ	Χ		Х	Χ	Х	Х
Complete Case Report Forms (CRFs)	Х						Χ					Х
Complete Daily Stool Diary		Х			Х			Х				
Complete Weekly Symptom Questionnaires (VAS)			Х	Х		Х	Χ		Х	Х	Х	Х
Randomization				Х								
Dispense study drug(s)				Х								
Return unused study drug(s)							Χ					

^{*}Women of childbearing potential

2.1 STUDY RATIONALE

Although rifaximin is very effective in treating IBS-D, symptom relapse is common, leading to the need for repeat treatments (7). A possible reason for the frequency of symptom relapse in IBS-D is the viscosity of the mucus layer in the small intestine (1, 2). This viscosity can render microbes in the mucus layer inaccessible, which may protect them from the effects of rifaximin, facilitating their regrowth and symptom recurrence. We recently showed that use of the reducing agent dithiothreitol (DTT), which can reduce the disulfide bonds linking mucin subunits (3), reduced viscosity and improved bacterial recovery in small intestinal aspirates (4). This led us to hypothesize that using rifaximin in combination with a mucolytic agent would make mucosal bacteria more accessible and thus improve the efficacy of rifaximin in treating IBS symptoms. In this study, we propose to test this hypothesis using rifaximin in combination with N-acetylcysteine (NAC), a mucolytic agent which is used in the management of chronic obstructive pulmonary disease (COPD) (5) and other conditions (6). Specifically, we will test and compare the efficacies of different does of rifaximin in combination with NAC vs. rifaximin alone in human subjects.

2.2 BACKGROUND

Irritable bowel syndrome (IBS) is one of the most common functional gastrointestinal disorders, affecting 11% of the world's population (8), and accounting for 50% of all gastrointestinal office visits (9). It is characterized by abdominal pain, cramping and bloating, accompanied by altered bowed habits. The major forms of IBS are diarrhea-predominant (IBS-D), constipation-predominant (IBS-C) and mixed IBS (IBS-M). IBS can be a chronic, long-term condition, with up to 57% of subjects who otherwise had normal bowel function continuing to have altered bowel function for at least 6 years after recovering from the initial acute illness (10). As a result, the health care costs of IBS have been estimated at over \$30 billion per year (11). Further, this results in serious quality of life implications, which have been likened to diabetes or heart disease, in young adults who should otherwise be productive and healthy (12).

There is significant bacterial involvement in IBS, particularly IBS-D. IBS-D can be precipitated by acute gastroenteritis, which is caused by infection with bacterial pathogens such as *Escherichia coli* (13), *Salmonella* (14), *Shigella* (15) and *Campylobacter jejuni* (16). In addition, there is now overwhelming evidence that small intestinal bacterial overgrowth (SIBO) contributes to the symptoms of IBS-D. SIBO is defined as the presence of excessive numbers of coliform bacteria (which are more typically found in the colon) in the small bowel. Initially, we demonstrated that IBS patients have a higher prevalence of SIBO (up to 84% of IBS subjects) based on lactulose breath testing (17, 18), and this was later corroborated via glucose breath test studies (19-21). A subsequent meta-analysis validated that IBS subjects were more likely to have an abnormal breath test (OR=9.64, CI=4.26-21.82) than controls (22). Additionally, two studies confirmed that IBS subjects have a greater number of coliform bacteria than healthy controls using small bowel culture (23, 24), the latter of which found SIBO in 60% of IBS-D subjects (24), and one of the first deep sequencing studies of the small bowel demonstrated increased levels of Proteobacteria species including *Escherichia* sp, *Klebsiella* sp, and *Aeromonas* sp in IBS subjects compared to healthy controls (25).

A key finding that underscored bacterial involvement in IBS-D was the identification of the role of

cytolethal distending toxin B (CdtB) (26, 27). The bacterial pathogens which cause acute gastroenteritis and trigger the development of IBS, *E. coli* (13), *Salmonella* (14), *Shigella* (15) and *C. jejuni* (16), all produce a common toxin, cytolethal distending toxin (Cdt). Cytolethal distending toxin interferes with the cell cycle, and disrupts functions of specific cells such as lymphocytes and epithelial cells which provide immunity and physical barriers to microbial pathogens (28-31). Cdt is a heterotrimer composed of three subunits, CdtA, CdtB, and CdtC (30, 32, 33), of which CdB is the active subunit. In a series of studies, we showed that through molecular mimicry, antibodies raised by the host against CdtB bind to a host protein in the gut wall, vinculin (26). Vinculin is a cell adhesion protein with important roles in cell migration and adherence as well as neuronal function, and vinculin knockout mice fail to develop a mature nervous system ⁹. Moreover, we showed that vinculin is expressed in the interstitial cells of Cajal (ICC), which play key roles in neuromuscular control of the gut and are decreased in IBS-D patients (34-39). These findings led to our identification of increased circulating levels of antibodies to CdtB and vinculin in IBS-D, and led ultimately to the development of a diagnostic test for diarrhea-predominant IBS using levels of these antibodies as clinical biomarkers.¹¹

Consistent with the above-described roles for bacteria in IBS-D, antibiotic treatment has become a mainstay in the treatment of IBS (40-43). Of these, rifaximin is the only antibiotic currently approved by the FDA for the treatment of IBS-D (44). Rifaximin is an oral, broad-spectrum antimicrobial agent that is minimally absorbed (99.6% retained in the gut), targets the gastrointestinal tract, and associated with a low risk of clinically relevant bacterial antibiotic resistance (43, 45-47). It is generally recognized as having no side effects in blinded comparisons that differ from placebo. In two identically designed, phase 3, double-blind, placebo-controlled trials (TARGET 1 and TARGET 2) of patients with IBS-D, 40.7% of patients treated with rifaximin 550 mg 3 times daily (TID) for 2 weeks experienced adequate relief of global IBS symptoms, compared with 31.7% of patients treated with placebo (P<0.001) (43). In addition, a greater percentage of rifaximin-treated than placebo-treated patients reported durable improvement in IBS-D symptoms for at least 10 weeks post-treatment (43).

Although rifaximin is very effective in treating IBS-D, symptom relapse is common, leading to the need for repeat treatments (7). A possible reason for the frequency of symptom relapse in IBS-D is the viscosity of the mucus layer in the small intestine, which may render microbes in the mucus layer inaccessible, protecting them from the effects of rifaximin (1, 2). In this study, we propose to test whether combining rifaximin with a clinically approved mucolytic agent, N-acetylcysteine (NAC), can result in improvement in stool form and reduction in stool frequency, as well as improved relief of clinical symptoms, in subjects with IBS-D.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

Risks of rifaximin

Rifaximin is an oral, minimally absorbed, broad-spectrum antimicrobial agent that targets the gastrointestinal tract and is associated with a low risk of clinically relevant bacterial antibiotic resistance (43, 45-47). It is generally recognized as having no side effects in blinded comparison that differ from placebo, and was approved for the treatment of IBS-D by the US Food and Drug Administration in May 2015 (48). In two identically designed, phase 3, double-blind, placebo-controlled trials (TARGET 1 and TARGET 2) of patients with IBS-D, 40.7% of patients treated with rifaximin 550 mg 3 times daily (TID) for 2 weeks experienced adequate relief of global IBS symptoms, compared with 31.7% of patients treated

with placebo (P<0.001) (43). In addition, a greater percentage of rifaximin-treated than placebo-treated patients reported durable improvement in IBS-D symptoms for at least 10 weeks post-treatment. A third phase 3, randomized, double-blind, placebo-controlled trial was subsequently performed to evaluate the safety and efficacy of repeat treatment with rifaximin (7). In this trial, 636 patients who responded to open-label rifaximin and later relapsed were randomly assigned to receive repeat treatment with rifaximin (n = 328) or placebo (n = 308). The percentage of responders was significantly greater with rifaximin than placebo (n = 308). The percentage of responders of responders for abdominal pain (n = 328) or placebo (n = 308). Significant improvements were also noted for prevention of recurrence, durable response, and bowel movement urgency, and adverse event rates were low and similar between groups (n = 328).

The safety of rifaximin for the treatment of IBS-D was evaluated in 3 placebo-controlled studies in which 952 patients were randomized to rifaximin 550 mg three times a day for 14 days. Across the 3 studies, 96% of patients received at least 14 days of treatment with rifaximin. In trials 1 and 2, 624 patients received only one 14-day treatment. Trial 3 evaluated the safety of rifaximin in 328 patients who received 1 open-label treatment and 2 double-blind repeat treatments of 14 days each over a period of up to 46 weeks. The combined population studied had a mean age of 47 (range: 18 to 88) years, of whom approximately 11% of the patients were ≥65 years old, 72% were female, 88% were white, 9% were black and 12% were Hispanic.

Common Adverse Reactions:

The adverse reaction that occurred at a frequency ≥2% in rifaximin-treated patients at a higher rate than placebo in trials 1 and 2 for IBS-D was:

• Nausea (3% rifaximin, 2% placebo)

The adverse reactions that occurred at a frequency ≥2% in rifaximin-treated patients (n=328) at a higher rate than placebo (n=308) in Trial 3 for IBS-D during the double-blind treatment phase were:

- ALT increased (rifaximin 2%, placebo 1%)
- Nausea (rifaximin 2%, placebo 1%)

Less Common Adverse Reactions:

The following adverse reactions, presented by body system, were reported in less than 2% of patients in clinical trials of traveler's diarrhea and IBS-D:

- Hepatobiliary disorders: Clostridium colitis
- Investigations: Increased blood creatine phosphokinase
- Musculoskeletal and connective tissue disorders: myalgia

Specific Populations: Pregnancy and Lactation

There are no available data on rifaximin use in pregnant women to inform any drug associated risks. Teratogenic effects were observed in animal reproduction studies following administration of rifaximin to pregnant rats and rabbits during organogenesis at doses approximately 0.9 to 5 times and 0.7 to 33 times, respectively of the recommended human doses of 600 mg to 1650 mg per day. In rabbits, ocular, oral and maxillofacial, cardiac, and lumbar spine malformations were observed. Ocular malformations were observed in both rats and rabbits at doses that caused reduced maternal body weight gain. Due to these potential risks, women of childbearing potential will be required to undergo pregnancy testing and a positive pregnancy test will result in exclusion from the study. All subjects (male and female) will be required to use an acceptable method of contraception throughout the study. In addition, there is no information regarding the presence of rifaximin in human milk, the effects of rifaximin on the breastfed

infant, or the effects of rifaximin on milk production. Due to these potential risks, nursing mothers will also be excluded from the study.

Risks of NAC

Labeled indications for the use of NAC are:

- (i) To treat acetaminophen overdose: Acetylcysteine is used to prevent or lessen hepatic injury, as it acts as a hepatoprotective agent by restoring hepatic glutathione, serving as a glutathione substitute, and enhancing the nontoxic sulfate conjugation of acetaminophen. 72-hour oral and 21-hour IV regimens are FDA approved.
- (ii) As a mucolytic agent: Acetylcysteine exerts its mucolytic action through its free sulfhydryl group, which opens the disulfide bonds and lowers mucus viscosity. This action increases with increasing pH and is most significant at pH 7 to 9. The mucolytic action of acetylcysteine is not affected by the presence of DNA. N-acetylcysteine has been demonstrated to cause a decrease in sputum consistency, to facilitate easier expectoration, and to increase sputum volume. Bronchial mucus is composed of over 95% water; however, the physical characteristics of the mucus are due to glycoproteins. These glycoproteins bind to each other by way of disulfide bonds and give the mucus viscosity. N-acetylcysteine ruptures these disulfide bonds causing depolymerization and a rapid decrease in mucus viscosity. It also produces an irritative bronchorrheic effect on the mucosa, stimulating mucociliary clearance; this irritative effect may cause bronchospasm, thus acetylcysteine is not recommended in asthmatics. Solutions for inhalation/oral administration or effervescent tablets may be used.

NAC is also available over the counter in 600 mg and 900 mg capsules to promote immune and respiratory system health.

This study will utilize the 600 mg NAC over-the-counter capsules (Nature's Blend brand, NDC 54629-4097-60).

Adverse Reactions

Common: Gastrointestinal disturbance, diarrhea, nausea, vomiting, fatigue, conjunctival irritation, skin

Other: Hypotension, anaphylaxis, asthma attacks, headache

Case reports

Photosensitivity not attributable to location, season, or concomitant medication; occurred among pulmonary fibrosis patients more frequently with acetylcysteine than placebo in combination with pirfenidone

Prohibited Drugs/Interactions

Nitroglycerin: Severe headache due to added vasodilation effect

Imipramine and escitalopram: NAC can reduce the minimum effective dose.

Additional risks

Additional risks associated with participation in this study include the following:

1) Risks of lactulose, which is administered during the lactulose breath test. These include bloating, abdominal discomfort, and/or diarrhea after drinking the lactulose, however these symptoms are usually mild.

2.3.2 KNOWN POTENTIAL BENEFITS

Potential benefits to subjects include improvements in clinical symptoms of abdominal pain, stool consistency, bloating, and bowel movement urgency.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Rifaximin is a minimally absorbed antibiotic that is recognized as having no side effects in blinded comparison that differ from placebo. Associated risks include an increased risk of nausea. NAC is also associated with risks as detailed above in section 2.3.1, which for the over-the-counter doses include gastrointestinal disturbance, diarrhea, nausea, vomiting, fatigue, conjunctival irritation and skin rash. IBS-D is associated with abdominal pain, cramping and bloating, and diarrhea, and can have extremely negative effects on quality of life in affected individuals. The maximum dose of rifaximin subjects will receive is one standard 14-day course for IBS-D (1650mg/day for 14 days). The maximum dose of NAC subjects will receive is 1800 mg per day for 14 days. The other risks of the study include mild risks for nausea following ingestion of the lactulose substrate for the breath tests. These low risks outweigh the value of the potential benefits in identifying whether a combination of rifaximin and NAC therapy can bring increased benefits in terms of relief from clinical symptoms to IBS-D patients.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR	
	D :	ENDPOINTS	
Primary	Primary		
To assess the efficacy of combined	1. Improvement in stool form and	These endpoints were	
Rifaximin and NAC therapy vs.	reduction in stool frequency from	chosen as they align	
Rifaximin alone in decreasing	baseline, as determined from stool	with the endpoints	
clinical symptoms in subjects with	diary data comparing rifaximin	chosen to assess	
IBS-D.	alone vs rifaximin and NAC	rifaximin efficacy in the Target 1 & 2 trials.	
	2. Decrease in severity of abdominal		
	pain from baseline, as determined		
	from weekly average visual analog		
	scale (VAS) scores, relative to		
	rifaximin alone.		
	Secondary		
	1. Improvement in urgency from	These endpoints were	
	baseline, as determined from	chosen as they align	
	weekly average VAS scores,	with the endpoints	
	relative to rifaximin alone	chosen to assess	
		rifaximin efficacy in the	
	2. Improvement in bloating from	Target 1 & 2 trials.	
	baseline, as determined from		
	weekly average VAS scores,		
	relative to rifaximin alone		

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
	3. Reduction of H ₂ on lactulose hydrogen breath test (LHBT) from baseline, relative to rifaximin alone	
Secondary (Exploratory)	Exploratory	
To determine the effects of rifaximin in combination with NAC on the gut microbiome	Changes in microbiome profiles from baseline, as determined by 16S rRNA gene sequencing	Rifaximin has been shown to have minimal effects on the gut microbiome. In this study, we will determine the effects of combining rifaximin with NAC on the gut microbiome, using stool samples
To determine whether artificial intelligence characterization of Bristol stool scores (via the Dieta App) for stool photos can be used to normalize stool scores	Normalization of stool scores from baseline	Subjective interpretation of stool scores is a challenge for studies. In this study, we will test whether using the novel Dieta App to characterize Bristol stool scores for stool photos can reduce this subjectivity and normalize stool scores

Primary Endpoints:

- 1. Improvement in stool form and reduction in stool frequency from baseline, as determined from stool diary data comparing rifaximin alone vs rifaximin and NAC
 - The proportion of subjects who achieve improvements in stool form from baseline each week during the Treatment and Follow-Up Phases, as determined from responses in the Daily Stool Diary, where stool is classed based on the Bristol Stool Form Scale as one of 7 types (from Type 1 [separate hard lumps] to Type 7 [entirely liquid]. Daily responses will be reviewed at the end of each Phase upon diary return and weekly average scores will be complied. The weekly average scores during the Treatment and Follow-Up Phases will then be compared to the weekly average scores at baseline (i.e. during the Run-In Phase) to determine change from baseline in weekly average score.

- The proportion of subjects who achieve reductions in weekly average number of bowel
 movements each week during the Treatment and Follow-Up Phases compared to baseline. Stool
 frequency is determined from responses in the Daily Stool Diary. Daily responses will be
 reviewed at the end of each Phase upon diary return and weekly average scores will be
 complied. The weekly average scores during the Treatment and Follow-Up Phases will then be
 compared to the weekly average scores at baseline to determine change from baseline in
 weekly average score.
- 2. Decrease in severity of abdominal pain from baseline, as determined from weekly average visual analog scale (VAS) scores, relative to rifaximin alone.
 - The proportion of subjects who achieve reductions in weekly average abdominal pain scores during the Treatment and Follow-Up Phases compared to baseline. Abdominal pain is scored both in the Daily Stool Diary (on a scale of 1 to 10, 10 being the worst) and in the Weekly Symptom Questionnaires (also on a scale of 1 to 10). Daily and weekly responses will be reviewed at the end of each Phase upon diary return and weekly average scores will be complied. The weekly average scores during the Treatment and Follow-Up Phases will then be compared to the weekly average scores at baseline to determine change from baseline in weekly average score.

The proportion of weekly and daily responders will also be determined. A subject will be categorized as a weekly responder if the subject is a weekly responder in both pain intensity and stool consistency. An Abdominal Pain Weekly Responder is defined as a patient who experiences a decrease in the weekly average of worst abdominal pain in the past 24 hours score of at least 30 percent compared with baseline. A Stool Consistency Weekly Responder is defined as a patient who experiences a 50 percent or greater reduction in the number of days per week with at least one stool that has a consistency of Type 6 or 7 compared with baseline.

A patient is categorized as a daily responder if the patient is a responder in both pain intensity and stool consistency. An Abdominal Pain Intensity Daily Responder is defined as a patient who experiences a decrease in worst abdominal pain in the past 24 hours score of at least 30 percent compared with baseline. A Stool Consistency Daily Responder is defined as a patient whose stool consistency is less than 5 for all bowel movements on that day or no bowel movement.

Secondary Endpoints:

- 1. Improvement in urgency from baseline, as determined from weekly average VAS scores, relative to rifaximin alone
 - The proportion of subjects who achieve reductions in weekly average urgency scores during the Treatment and Follow-Up Phases compared to baseline. Urgency is scored in the Weekly Symptom Questionnaires (on a scale of 1 to 10, 10 being the worst). Weekly responses will be reviewed at the end of each Phase upon diary return and weekly scores during the Treatment and Follow-Up Phases will be compared to the weekly scores at baseline, to determine change from baseline in weekly average score.

- 2. Improvement in bloating from baseline, as determined from weekly average VAS scores, relative to rifaximin alone
 - The proportion of subjects who achieve reductions in weekly average bloating scores during the Treatment and Follow-Up Phases compared to baseline. Urgency is scored in the Weekly Symptom Questionnaires (on a scale of 1 to 10, 10 being the worst). Weekly responses will be reviewed at the end of each Phase upon diary return and weekly scores during the Treatment and Follow-Up Phases will be compared to the weekly scores at baseline, to determine change from baseline in weekly average score.

The proportion of overall bloating responders will also be determined. An overall bloating responder is defined as a subject with a weekly bloating response in at least 50% of the weeks of treatment and follow-up. A weekly bloating response is defined as a weekly average bloating score of at least 30% improvement compared to baseline.

- 3. Reduction of H₂ on lactulose hydrogen breath test (LHBT) from baseline, relative to rifaximin alone
 - Change from baseline in the area-under-the-curve (AUC) of breath H₂ production, based on the 120-minute lactulose breath test. Test AUCs at Visits 3 and 4 will be compared to those at Visit 1.

Exploratory endpoints:

- 1. Changes in microbiome profiles from baseline, as determined by 16S rRNA gene sequencing
 - Microbiome profiles will be determined in stool samples provided on Visits 2, 3 and 4. These
 analyses will be performed in batch at the end of the study and the profiles at Visits 3 and 4 will
 be compared to those at Visit 2.
- 2. Normalization of stool scores from baseline
 - Daily responses from the Dieta app will be reviewed at the end of each Phase and weekly
 average scores will be complied. The weekly average scores during the Treatment and FollowUp Phases will then be compared to the weekly average scores at baseline (i.e. during the RunIn Phase) to determine change from baseline in weekly average score. These data will then be
 compared to the data obtained from the Daily Stool Diaries and Weekly Symptom
 Questionnaires.

4 STUDY DESIGN

4.1 OVERALL DESIGN

The proposed study is a prospective proof-of-concept double-blind (to dose of NAC) clinical trial to determine the efficacy of combined rifaximin and NAC therapy vs. rifaximin alone in decreasing clinical symptoms in subjects with IBS-D.

The study is a single-site, open-label trial which will consist of 3 phases:

- 1) Run-In Phase: Eligible subjects with IBS-D who consent to participate will be recruited (N=45). After provision of informed written consent, these subjects will undergo screening and baseline testing as described in the Schedule of Activities and will complete a daily stool diary for 14 days as well as weekly symptom questionnaires.
- 2) <u>Treatment Phase:</u> Subjects will be randomized in (blocks of 3) to one of 3 study arms and will receive either:
 - a. Rifaximin 550 mg by mouth three times daily for 14 days (N=15)

OR

b. Rifaximin 200 mg plus placebo by mouth three times daily for 14 days (N=15).

OR

c. Rifaximin 200 mg plus N-acetylcysteine (600mg) by mouth three times daily for 14 days (N=15)

Subjects will complete a daily stool diary for 14 days of treatment as well as weekly symptom questionnaires.

c) <u>Follow-Up Phase:</u> Upon completion of treatment, subjects will undergo repeat testing as at baseline. Subjects will then complete a daily stool diary for 28 days as well as weekly symptom questionnaires. At the end of this phase, subject will complete an end-of-study visit with additional repeat testing as described in the Schedule of Activities.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

It is well known that treatment of IBS-D with rifaximin is effective and now FDA-approved. However, only 44% of subjects improved with rifaximin treatment. Although what is unique about rifaximin is its 'one-and-done' treatment effect, this is only seen in 36% of subjects who respond to this drug. As such, there is room for improvement with rifaximin. In recent studies, we have shown that the most predominant bacteria in the bacterial overgrowth associated with IBS are *E. coli* and *Klebsiella*. Rifaximin is highly effective in treating these two organisms. However, we have since learned that the majority of these excessive organisms in IBS are found in the small intestinal mucus layer. Since rifaximin is not soluble in mucus, it cannot penetrate and affect bacteria within the mucus layer. Our hypothesis is that the addition of a mucolytic like NAC will allow the penetration of rifaximin into the mucus by first solubilizing rifaximin and secondly liquifying the mucus. This may allow for two important effects. One is a reduction in the necessary dose of rifaximin necessary to treat IBS, and the other is improved efficacy. Both of these will be tested in this trial.

In addition, this study has 2 exploratory goals: To determine the effects of rifaximin in combination with NAC on the gut microbiome, and to determine whether artificial intelligence characterization of Bristol stool scores (via the Dieta App) for stool photos can be used to normalize stool scores. Rifaximin is a minimally-absorbed antibiotic and has been noted in several studies to have minimal effects on the gut microbiome (49, 50), and this is one of the advantages of using rifaximin. Therefore in this study, we also propose to determine the effects of combined treatment with rifaximin and NAC vs rifaximin alone or rifaximin plus placebo on the gut microbiome, by comparing the gut microbial populations in stool samples obtained from all 3 groups.

Additionally, the Dieta mobile app will be provided to subjects to assess the first objective measurement of Bristol stool score using an App. This app has not been tested in clinical trial previously and thus this is part of an exploratory endpoint. The Dieta mobile app allows patients to track their dietary intake, clinical symptoms and stool form. It includes a stool image capture feature and computer vision technology that objectively classifies stool consistency based on Bristol stool score. Traditionally, patients assess stool form and consistency using the Bristol stool score and chart, which classifies stool into one of seven types. A challenge with this is that the assessment is subjective, and may vary from patient to patient. The Dieta app allows subjects to take photographs of their stool and have it classified for them. The use of Dieta in this study will allow us to determine whether using the Dieta App can reduce this subjectivity and normalize stool scores, and thus could be useful in future studies.

4.3 JUSTIFICATION FOR DOSE

Rifaximin is currently approved by the FDA for the treatment of IBS-D and for traveler's diarrhea. The dosing for IBS-D is 550 mg per mouth three times daily for 14 days, and the dosing for traveler's diarrhea is rifaximin 200 mg per mouth three times daily for 3 days.

The proposed study design will compare the following doses:

- Rifaximin 550 mg per mouth three times daily, which is the standard FDA-approved dose for the treatment of IBS-D. This dose will be the comparator against which the two other combinations will be assessed.
- Treatment with 200 mg rifaximin alone per mouth three times daily which is the standard FDAapproved dose for the treatment of traveler's diarrhea, but is not expected to be effective against IBS-D. This will be given in combination with a placebo as a control.
- Combined treatment with the mucolytic agent NAC at 600 mg by mouth 3 times daily and 200 mg rifaximin three times daily. The 600 mg dose of NAC was chosen because it is available over the counter as a dietary supplement, and as such is expected to be safe, although it is acknowledged that the effects of taking the two drugs together is unknown. The primary goal of the study is to determine if taking NAC in addition to the 200 mg dose of rifaximin will, by virtue of breaking up the small intestinal mucus and making microbes in the mucus accessible to the rifaximin, will render the 200 mg dose as effective as the standard 550 mg dose, allowing us to give a lower dose of antibiotic. The 200 mg dose was chosen rather than another dose less than 550 mg as it is commercially available. Additional doses may be tested in further studies.

The maximum dose of rifaximin subjects will receive is one standard 14-day course for IBS-D (1650mg/day for 14 days). The maximum dose of NAC for subjects will be 1800 mg per day for 14 days.

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA), Section 1.3.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

The inclusion criteria for this study are as follows:

- 1) Male or female subjects aged 18-75 years old inclusive
- 2) Meet Rome IV criteria for IBS-D and, in order to progress to treatment phase, meet the following:
 - A. Has abdominal pain, on average, ≥ 1 day per week in previous 3 months, associated with ≥ 2 of the following: pain related to defecation, pain associated with a change in stool frequency, pain associated with a change in form (appearance) of stool. Onset of clinical symptoms have occurred for at least 6 months.
 - B. Has >25% of abnormal bowel movements with Bristol stool form types 6 or 7 (loose, watery stool) and <25% of abnormal bowel movements with Bristol stool form types 1 or 2 (hard, lumpy stool).
- 3) Colonoscopy must have been completed for subjects over 45 years of age
- 4) Subjects are capable of understanding the requirements of the study, are willing to comply with all the study procedures, and are willing to attend all study visits.
- 5) All subjects (male and female) shall agree to use an acceptable method of contraception throughout their participation in the study. Acceptable methods of contraception include:
 - a) double barrier methods (condom with spermicidal jelly or a diaphragm with spermicide),
 - b) hormonal methods (e. g. oral contraceptives, patches or medroxyprogesterone acetate) together with one of the barrier methods described above,
 - c) an intrauterine device (IUD) with a documented failure rate of less than 1% per year.
 - d) Abstinence or partner(s) with a vasectomy may be considered an acceptable method of contraception at the discretion of the investigator.
 - e) Female subjects who have been surgically sterilized (e.g. hysterectomy or bilateral tubal ligation) or who are postmenopausal (total cessation of menses for >1 year) will not be considered "females of childbearing potential".

All subjects will provide Institutional Review Board (IRB)-approved informed written consent prior to beginning any study-related activities.

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

- 1) Use of any oral antibiotics in the last two months
- 2) Subjects with history of intestinal surgery (except appendectomy or cholecystectomy)
- 3) Subjects with known pelvic floor dysfunction
- 4) Pregnancy

- 5) Nursing mothers
- 6) Poorly controlled/uncontrolled significant medical condition that would interfere with study procedures
- 7) History of bowel obstruction
- 8) History of inflammatory bowel disease or celiac disease
- 9) History of HIV
- 10) Cirrhosis
- 11) IBS-C/chronic idiopathic constipation
- 12) Poorly controlled diabetes or thyroid disease
- 13) History of anorectal radiation/surgery
- 14) History of prostatitis
- 15) Known allergy or hypersensitivity to rifaximin, rifamycin or NAC
- 16) Current treatment with eluxadoline or opiates
- 17) Current treatment with warfarin or cyclosporine
- 18) Current treatment with nitroglycerin, Imipramine or escitalopram
- 19) Patients with symptomatic bowel obstruction, diverticulitis and/or adhesions
- 20) Patients with asthma, due to risk of bronchospasm from NAC
- 21) Patients with any history of anaphylaxis
- 22) Patients with history of hepatic disease, including patients with elevated liver enzyme levels (aspartate aminotransferase [AST], alanine aminotransferase [ALT] or alkaline phosphatase [ALP]) that are ≥ 1.5 times the upper limit of normal (ULN) that have not been resolved within the 4 weeks prior to consent and/or are present at the Screening Visit laboratory assessment.
- 23) Patient has any abnormal laboratory results or physical examination findings deemed clinically significant by the investigator during the Screening Visit or in the Screening Visit laboratory assessment.
- 24) Patients with hematochezia, including occult blood as determined by Fecal Occult Blood Test (FOBT), at Screening Visit.

Development of any of the exclusion criteria during the study will be considered a basis for subject discontinuation.

5.3 LIFESTYLE CONSIDERATIONS

Not applicable

5.4 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of a failure to meet Rome criteria may be rescreened if symptoms change in time. Rescreened participants should be assigned the same participant number as for the initial screening.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

Recruitment

Adult patients (age 18-75) with IBS-D (diagnosed based on Rome IV criteria (51)) will be assessed for eligibility to participate in this study. Potential subjects will be identified through the GI Motility Clinic at Cedars-Sinai Medical Center or through one of the methods described below), and will be assessed for eligibility to participate in this study using the Inclusion/Exclusion Criteria.

Potential subjects will be identified and recruited using one or more of the following approaches:

- 1) After a review of medical records of patients under the care of one or more of the study investigators
- 2) Be identified and approached during an inpatient or outpatient clinical visit by a member of the research team
- 3) Be identified by their treating physicians and referred to the researchers. Patients' private and identifiable information will not be shared with the researchers prior to receiving permission from the patient.
- 4) Using an IRB-approved cohort building tool. Initial contact of potential subjects will be made through a treating physician.
- 5) Self-referral in response to advertisements

Pre-screening may be conducted over the phone or using an online screening tool. Subjects will be given a complete explanation of the study and given/emailed the consent form to review at their leisure and discussed with their doctor, family and friends before being asked to sign a consent form. Patients will be provided time to ask questions, and it will be re-iterated that their participation is voluntary and that there is no obligation to participate.

Retention

To enhance participant retention, weekly phone visits will be scheduled with subjects during all three phases of the study (Run-In, Treatment and Follow-Up) to assist with completing the Weekly Symptom Questionnaires and to provide subjects with the opportunity to ask questions or relay concerns. In addition, the MAST program phone number and study staff emails will be provided to patients, and they will be made aware that they can call or email to ask any questions.

The daily stool diaries and weekly symptom questionnaires can either be filled online using REDCap, a secure, web-based application for building and managing online surveys and databases with which Cedars-Sinai is partnered, or completed in paper format, to accommodate different patient preferences. In addition, the Dieta mobile app will be provided to subjects for the objective assessment of Bristol stool score. This app has not been tested in clinical trial previously and thus this is part of an exploratory endpoint.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

The study interventions are as follows:

- 1) Rifaximin 550 mg by mouth tid
- 2) Rifaximin 200 mg plus placebo by mouth tid
- 3) Rifaximin 200 mg plus NAC 600 mg by mouth tid

Rifaximin is currently approved by the FDA for the treatment of IBS-D and for traveler's diarrhea. The dosing for IBS-D is 550 mg per mouth three times daily for 14 days, and the dosing for traveler's diarrhea is rifaximin 200 mg per mouth three times daily for 3 days.

Labeled indications for NAC include its use as a mucolytic agent in patients with abnormal, viscid, or inspissated mucous secretions in conditions such as: chronic bronchopulmonary disease such as chronic emphysema, acute bronchopulmonary disease such as pneumonia and bronchitis, and pulmonary complications of cystic fibrosis, amongst others. NAC is available over the counter in 600 mg and 900 mg capsules to promote immune and respiratory system health, although such over-the-counter uses are not regulated by the FDA.

6.1.2 DOSING AND ADMINISTRATION

During the treatment phase of the study, subjects will be randomized to receive the study interventions as follows:

1) Rifaximin 550 mg by mouth three times daily for 14 days

OR

2) Rifaximin 200 mg plus placebo by mouth three times daily for 14 days

OR

3) Rifaximin 200 mg plus NAC 600 mg by mouth three times daily for 14 days

Subjects will be instructed not to take the study drugs with meals. In addition, subjects in Groups 2 and 3 will be instructed to take the study drugs 2 hours apart, due to potential effects of NAC on antibiotics.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

Both doses of rifaximin (550 mg, 220 mg) will be supplied to the study team by Cedars-Sinai research pharmacy. Rifaximin is approved by FDA for use in patients with IBS-D, hepatic encephalopathy and traveler's diarrhea. NAC (600 mg) will also be obtained from the Cedars-Sinai research pharmacy. Placebos will also be generated by the Cedars-Sinai research pharmacy.

Subjects will be provided medication packs sufficient for 14 days. On day 0 (see Schedule of Activities), a study team member will dispense the packs to the subject. For the purpose of accountability, all unused packs dispensed to the subject should be returned to the site on Visit 3 (see Schedule of Activities). In order to maintain compliance with federal regulations, records of the disposition of drug will include: dates dispensed, quantity, use by the subject and dates of returns. 90% study drug compliance will be considered acceptable.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

The 550 mg tablet of rifaximin will be the standard oval salmon colored tablet. The 200mg tablet of rifaximin will be the standard round salmon colored tablet. The NAC will be a powder within a coated capsule. The placebo to NAC will contain an inert powder (carrier) with a capsule matching the NAC capsule.

On Visit 2 (day 0, see Schedule of Activities), subjects will be provided medication packs sufficient for 14 days.

For all study investigational product, a system of investigational product numbering in accordance with all requirements of current Good Manufacturing Practice (GMP) will be used. This will ensure that for each subject, any measured quantity of study investigational product can be identified and traced back to the original pack of medication.

Lists linking all numbering levels will be maintained by the institutions in charge of investigational product packaging including the Sponsor.

Each pack will bear a label which includes, but is not limited to, the following information:

- Product: Rifaximin 550 mg <u>OR</u> Rifaximin 200 mg + placebo <u>OR</u> Rifaximin 200 mg + NAC 600 mg. This information will be included on a tear-off portion of the label and will be removed before dispensing to the patient.
- Kit Number
- Quantity
- Instructions for taking product
- Storage conditions: Store packets/kit at 20-25°C (68-77°F): excursions permitted between 15-30°C (59-86°F)
- Manufacturer

In addition, each kit label will contain an area for the site to write the following information:

- Subject initials
- Subject ID Number
- Date Dispensed

Date Returned

6.2.3 PRODUCT STORAGE AND STABILITY

All investigational product will be kept in a locked area with limited access to study staff only. The locked area will be maintained under controlled temperature conditions at 20-25°C (68-77°F); excursions are permitted to 15-30°C (59-86°F).

6.2.4 PREPARATION

Not applicable.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Cedars-Sinai pharmacy will perform the blinding and randomization and hold the codes associated with assigned drug assignment of NAC. Since there are three groups, the subjects will follow a randomization table and randomized in blocks of 3 to maintain an even distribution of randomized subjects.

6.4 STUDY INTERVENTION COMPLIANCE

REDCap provides an alert system to the patient to enhance compliance with the primary endpoint. Study personnel will track compliance and call patients in the instance of a deficiency for more than 2 days.

Study intervention compliance will also be assessed via reviews of the daily stool diaries and weekly symptom questionnaires, which may be completed in paper form or filled online using REDCap, and through reviews of associated data captured using the Dieta app. In addition, weekly phone visits will be scheduled with subjects during all three phases of the study (Run-In, Treatment and Follow-Up) to assist with completing the Weekly Symptom Questionnaires and promote compliance. Lastly, all unused packs dispensed to the subject are to be returned to the site on Visit 3, which will provide an additional opportunity to assess compliance.

6.5 CONCOMITANT THERAPY

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the Case Report Form (CRF) are concomitant prescription medications, over-the-counter medications and supplements.

6.5.1 RESCUE MEDICINE

Although the use of rescue medications is allowable at any time during the study, the primary symptom in subjects with IBS-D is diarrhea. As such, the study will allow for use of the anti-diarrheal, Imodium (loperamide), for the acute treatment of uncontrolled diarrhea where symptoms are severe enough to affect the subject's Activities of Daily Living (ADLs) on a given day as follows:

Loperamide at a unit dose of 2 mg may be taken once approximately every 6 hours with the

following guidelines:

- No more than 4 unit doses over a continuous 24-hour time period (8 mg/day)
- No more than 7 unit doses over a continuous 48-hour time period (14 mg over 2 days)
- No more than 11 unit doses over a continuous 7-day time period

Study subjects are encouraged to avoid Imodium except when symptoms are severe. If rescue medications are used, this needs to be documented. Days in which Imodium is used will be considered a failed day.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Subjects will be considered for discontinuation of the study in the following circumstances:

- a. Subjects have a worsening of symptoms that are intolerable and as a result the subjects expresses a desire to withdraw from the study based on these symptoms
- b. Subjects experiences a serious adverse event (SAE) and the study monitor, investigators or designee feels that participation should be stopped. SAEs resulting in withdrawal of a patient could include hepatoxicity, *C. difficile* enterocolitis, bronchospasm, diarrhea and hypersensitivity reactions.
 - i. Hepatoxicity: Subjects who fit Common Terminology Criteria for Adverse Events (CTCAE) Grade 3 for Alkaline phosphatase, Alanine aminotransferase, Aspartate aminotransferase, Total bilirubin, GGT, Hepatic failure or Portal hypertension.
 - ii. *c. difficile* enterocolitis: Subjects presenting with worsening diarrhea affecting Activities of Daily Living (ADLs) or bloody stools will be tested for *C. difficile*.
 - iii. bronchospasm and hypersensitivity reactions: subjects experiencing CTCAE grade 3 for allergic reactions and anaphylaxis, including bronchospasm, with or without urticaria, parenteral intervention indicated, allergy-related edema/angioedema; hypotension will be withdrawn
 - iv. diarrhea: Subjects experiencing severe worsening diarrhea that interferes with ADLs and is refractory to rescue therapy and/or requires hospitalization for hydration
- c. Subject severely violates the study protocol or fails to complete the necessary steps in the trial.
- d. Subjects clinical laboratory assessments (including liver enzymes) are found to be abnormal. Specifically:
 - i. ALT or AST >8 x the ULN
 - ii. ALT or AST > 5 x ULN for more than 2 weeks
 - iii. ALT or AST > 3 x ULN **and** (total bilirubin > 2 x ULN or international normalized ratio>1.5)
 - iv. ALT or AST > 3 x ULN x ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (>5%).

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Pregnancy
- Significant study intervention non-compliance
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation

The reason for participant discontinuation or withdrawal from the study will be recorded on the Case Report Form (CRF). Subjects who sign the informed consent form and are randomized but do not receive the study intervention may be replaced. Subjects who sign the informed consent form, and are randomized and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

Data from subjects who discontinue or are withdrawn from the study will be included up until the last observation. The primary analysis will be conducted using the observed values. Patients must have no more than 2 missed reporting days among the past 7 days during an individual week to qualify as having improvement and hence classified as a responder. If fewer than 5 days are reported within a given week, then the patient is assumed a non-responder. For missing data, two sensitivity analyses will be conducted by using the multiple imputation and last observation carried forward (LOCF) methods. For secondary and exploratory analyses, for the responder endpoints, the analysis will be conducted by imputing the missing data as "non-responders". A sensitivity analysis will be conducted by imputing the missing data as "responders".

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for the follow-up visit immediately after cessation of therapy (Visit 3) or the visit 1 month after cessation of therapy (Visit 4).

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 5 days and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every
 effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary,
 a certified letter to the participant's last known mailing address or local equivalent methods).
 These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

Data from subjects who are lost to follow-up will be included up until the last observation.

8.1 EFFICACY AND SAFETY ASSESSMENTS

1) Demographics and medical history

The study staff will record the subject's sex, date of birth, and ethnic origin. Medical history (i.e. previous diagnoses, diseases or surgeries) will be collected. Findings will be recorded in REDCap via the medical history questionnaire (see Appendix 1). Concomitant medications will also be reviewed

2) Physical exam including vital signs

Complete physical exams will be performed by the principal investigator or a physician co-investigator. The general examination will include evaluation of the head, ears, eyes, nose, throat, endocrine, cardiovascular, respiratory, abdomen, skin, neurological, extremities, and musculoskeletal systems. Vital sign measurements, including height, body weight, blood pressure, radial pulse rate, respiratory rate, and temperature will be recorded after the subject has been semi-supine for at least five minutes prior to the test. Abnormalities noted at baseline (Visit 1) will be recorded as medical history. Any clinically significant change from baseline will be recorded as an adverse event.

3) Urine pregnancy test

Subjects who are women of childbearing potential will be asked to provide a urine sample for pregnancy testing. Pregnancy will be considered an Exclusion Criterion.

4) Lactulose breath test

Subjects will be asked to undergo a 24-hour preparatory period just prior to the test. Subjects will maintain a bland diet during the first 12 hours and fast during the remaining 12 (see Appendix 2). A baseline breath sample will be collected by exhaling into disposable collection bag with a volume of 750 mL. Subjects will then consume 10 g lactulose dissolved in water. Subsequent breath samples will be collected every 15 minutes over the remainder of the 120-minute test. Collected breath samples will be batched for analysis. The samples will be analyzed for hydrogen, methane, carbon dioxide, and hydrogen sulfide levels.

5) Clinical Symptom Questionnaires (VAS)

A baseline symptom questionnaire (see Appendix 3) will be completed. Symptom severity is recorded using a visual analog scale (VAS) on a scale of 0 (no symptom) to 100 (maximum symptom). This can either be filled online using REDCap, a secure, web-based application for building and managing online surveys and databases with which Cedars-Sinai is partnered, or completed in paper format.

6) Blood draw

Blood will be drawn by a trained research nurse and collected for complete blood count (CBC), complete metabolic panel (CMP), erythrocyte sedimentation rate (ESR), and, in women of childbearing potential, for serum pregnancy testing. Laboratory analyses will be performed in the central clinical laboratory at Cedars-Sinai Medical Center. Reference ranges will be supplied by the clinical laboratory and used by the investigator to assess the laboratory results for clinical

significance and pathological changes. An additional blood sample will be drawn for exploratory cytokines, which will be measured in the MAST research laboratory at Cedars-Sinai Medical Center.

7) Stool collection for Fecal Occult Blood Test

Subjects will be provided with a Hemoccult® Sensa® Single Slide Fecal Occult Blood Test (FOBT) kit and asked to provide a stool sample or place a stool smear on the test kit slide during the screening visit if possible. If this is not possible, subjects will be provided with the kit and instructions and asked to mail the sample back to the investigators as soon as possible (return kits will be provided). Analysis will be performed in the central clinical laboratory at Cedars-Sinai Medical Center.

8) Stool collection (optional)

At the end of the study visit, subjects will be provided with a stool collection kit and asked to obtain a stool sample on the day prior to their next visit. Subjects will be instructed to store the sample in the refrigerator and to return it to study staff on their visit the following day. The provision of a stool sample is optional and will be used for exploratory analyses of the effects of combined rifaximin and NAC on the gut microbiome.

9) Daily stool diary

Subjects will be asked to complete a daily stool diary (see Appendix 4) at home, using the Bristol Stool Chart (see Appendix 4) as a visual aid, either online using REDCap or in paper format. Diaries completed in paper format will be returned to study staff on their next study visit. Subjects will also be asked to use the Dieta mobile application, which includes a stool image capture feature and computer vision technology that objectively classifies stool consistency.

10) Weekly symptom questionnaires

Subjects will be asked to complete weekly symptom questionnaires (as in Appendix 3) at home, either online using REDCap or in paper format. Questionnaires completed in paper format will be returned to study staff on their next study visit. Again, subjects will be asked to use the Dieta mobile application to characterize stool form using artificial intelligence.

11) Phone visits

A study staff member will schedule a phone visit with subjects on the days they are to complete the weekly symptom questionnaires.

8.2 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.2.1 DEFINITION OF ADVERSE EVENTS (AE)

An Adverse Event (AE) will be a side effect that is an event reported by the subject that may be related to the study drugs (rifaximin and/or NAC) but may or may not prevent continuation of the trial (examples include body aches, loose stool).

8.2.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

A <u>Serious Adverse Event (SAE)</u> will be an event report by a subject during the trial may or may not be deemed related to study drug(s) that forces discontinuation of the trial (examples include allergic reaction, etc.).

An SAE is classified as any untoward medical occurrence that, at any dose, meets any of the following criteria:

- Results in death
- Is life-threatening
 - The term 'life-threatening' in the definition refers to an event in which the patient was at risk of death at the time of the event, it does not refer to an event which hypothetically might have caused death if it were more severe
- Requires inpatient hospitalization or prolongation of existing hospitalization
 - A hospitalization or prolongation of hospitalization will not be regarded as an SAE if at least one of the following exceptions is met:
 - The admission results in a hospital stay of less than 12 hours.
 - The admission is pre-planned.
 - The admission is not associated with an AE.
 - Results in persistent or significant disability / incapacity.
 - Disability means a substantial disruption of a person's ability to conduct normal life's functions.
 - Is a congenital anomaly/birth defect.
 - Is another medically important serious event as judged by the investigator.

8.2.3 CLASSIFICATION OF AN ADVERSE EVENT

8.2.3.1 SEVERITY OF EVENT

The severity of AEs will be graded as follows:

- Mild usually transient in nature and generally not interfering with normal activities
- Moderate sufficiently discomforting to interfere with normal activities
- Severe prevents normal activities

8.2.3.2 RELATIONSHIP TO STUDY INTERVENTION

The assessment of the relationship of an AE to the administration of the study drug(s) is a clinical decision based on all available information at the time of the completion of the source documentation of the AE or of the SAE form, if applicable.

An assessment of "Not" or "Unlikely" would include (Note: "Unlikely" is considered not related to the study drug(s)):

- The existence of a clear alternative explanation, e.g., mechanical bleeding at surgical site;
 or
- Non-plausibility, e.g., the subject is struck by an automobile when there is no indication that the study drug(s) caused disorientation that may have caused the event; cancer developing a few days after the first therapy administration.

An assessment of "Possible" or "Related" indicates that there is a reasonable suspicion that the AE is associated with the use of the study drug(s).

Factors to be considered in assessing the relationship of the AE to the study drug(s) include:

- The temporal sequence from administration of the study drug(s): The event should occur after the therapy is given. The length of time from therapy exposure to event should be evaluated in the clinical context of the event.
- Recovery of discontinuation (de-challenge), recurrence on re-introduction (re-challenge): Subject's response after discontinuation of the study drug(s) (de-challenge), or response after re-introduction of the study drug(s) (re-challenge) should be considered in the view of the usual clinical course of the event in question.
- Underlying, concomitant, intercurrent diseases: Each report should be evaluated in the context of the natural history and course of the disease being treated and any other disease the subject may have.
- Concomitant medication or treatment: The other therapies the subject is taking or the treatment the subject receives should be examined to determine whether any of them may be suspected to cause the event in question.
- Concurrent study procedures: Study procedures should also be considered as possible causes of an AE. Invasive procedures such as biopsies have their own profile of expected AEs and the Investigator should consider these.

The Investigator will make a separate and independent assessment of every AE's relationship not just to the study the study drug(s), but also to the subject's pre-existing medical condition(s).

8.2.3.3 EXPECTEDNESS

Expected adverse effects include the side effects observed in previous clinical trials, which included nausea (for rifaximin) and gastrointestinal disturbance, diarrhea, nausea, vomiting, fatigue, conjunctival irritation, and skin rash (for NAC). While some of these side effects occurred in more than 1% of subjects, in the case of rifaximin these were not more common than placebo.

8.2.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

All AEs and SAEs must be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or the subject is lost to follow-up. The investigator is responsible to ensure that follow-up includes any supplemental investigations as may be indicated to elucidate as completely as practical the nature and/or causality of the AE or SAE. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

8.2.5 ADVERSE EVENT REPORTING

All adverse events will be reported to a data safety monitor who is independent of the study. The assessment of the relationship of an AE to the administration of the study drug(s) is a clinical decision based on all available information at the time of the completion of the source documentation of the AE or of the SAE form, if applicable.

8.2.6 SERIOUS ADVERSE EVENT REPORTING

Serious adverse events must be reported promptly to Safety Monitor once the investigator determines that the event meets the protocol definition of an SAE. Suspected Unexpected Serious Adverse Reactions (SUSARs) that are fatal or life-threatening will be reported to the FDA within 7 days. All other SUSARs will be reported within 15 days, according to 21 CFR 312.32.In addition, AEs and SAEs that are unexpected and caused directly by the research study will be reported to the Institutional Review Board within 10 days.

Prompt reporting of a SAE requires:

- Completion and transmission of SAE information to the Sponsor within 24 hours of the investigator's knowledge of the event. The SAE It is very important that the investigator provide his/her assessment of causality to investigational product at the time of initial reporting of the SAE. SAE information should include at a minimum:
 - AE description (diagnosis)
 - Onset Date
 - Resolution date
 - Duration
 - Seriousness (Fatal, Life Threatening, Hospitalization require or prolonged, Congenital anomaly, Disabling/Incapacitating, Other describe)
 - Contributing factors (underlying disease being studied, concurrent illness specify if yes, concurrent therapy – specify if yes, other - describe)
 - List relevant medical conditions (past or current), include onset and resolution dates if known
 - List any medications taken to treat this SAE
 - Provide any relevant diagnostic procedures and laboratory results
 - Add a narrative/comments providing a textual description of the SAE including chronological presentation and evaluation of the SAE, associated signs and symptoms, treatment of the SAE and outcome of the SAE.

Prompt reporting of additional information for previously reported SAEs should follow the same reporting timeframe as initial reports and will be reported using MedWatch.

8.2.7 REPORTING EVENTS TO PARTICIPANTS

Not applicable

8.2.8 EVENTS OF SPECIAL INTEREST

Not applicable

8.2.9 REPORTING OF PREGNANCY

Pregnancies detected during study conduct should be promptly reported to Safety Monitor as soon as the investigator is notified. should be notified via fax of any updates on the status of the pregnancy as

soon as the information becomes available by update and/or amendment of the initial pregnancy reporting form. Pregnancies will be followed to term and information will be collected on the pregnancy outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, abnormalities, or complications.

8.3 UNANTICIPATED PROBLEMS

8.3.1 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

Not applicable

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

This is a pilot proof of concept study. It is well published that the rate of eradication of small intestinal bacterial overgrowth in IBS-D subjects with rifaximin at a dose of 550mg tid for 14 days is approximately 60%. Studies have further shown that the rate of eradication is 50% less with half this dose. The equivalent tablet to approximate this half dose is 200mg tid for 14 days. In this pilot proof the hope is that the addition of NAC provides a non-superiority. In other words, the addition of NAC to the lower less effective dose of rifaximin will be at least as effective as the larger 550mg dose.

9.2 SAMPLE SIZE DETERMINATION

Even though we consider this a pilot study, we have calculated the power using the independent samples (2-sample) t test. We are comparing the large dose (550mg) to the low dose (200mg) and this difference would modestly be 20 points with a SD of 18. If the true difference in the experimental and control means is 20, we will need to study 14 550mg subjects and 14 200mg subjects to be able to reproduce previous data with probability (power) 0.8. In the NAC plus rifaximin 200mg (low dose group) we expect no difference from the high dose group (equivalency) but also need 14 subjects. Accounting for dropouts, we will recruit 15 subjects per arm. The Type I error probability associated with this test of this null hypothesis is 0.05.

The POWER Procedure
Two-Sample t Test for Mean Difference

Fixed Scenario Elements

Distribution Normal Method Exact Mean Difference 20 Standard Deviation 18 **Nominal Power** 8.0 Number of Sides 2 Null Difference 0 Alpha 0.05

Computed N per Group

Actual Power 0.808 N per Group 14

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention. The following consent materials are submitted with this protocol:

Consent form with HIPAA and details of optional sub-study

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study. As the questionnaires for this study are not standardized and have not been translated into other languages, non-English speakers will not be included in this study.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable

cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).

Individual stopping criteria

If any subject demonstrates (i) clinical signs of potential serious liver injury, hepatotoxicity, and/or hyperbilirubinemia jaundice, or (ii) a hypersensitivity reaction determined by the investigator to be associated with treatment, (iii) determined to have *C. difficile* as a result of the treatment, (iv) bronchospasm, (v) worsening diarrhea interfering with ADLs that is refractory to rescue therapy and/or requires hospitalization for hydration, that subject should be withdrawn from therapy.

An increase in liver enzymes to > 3x ULN in patients who started the study with baseline values within normal limits will be followed by repeat testing within 48 to 72 hours to confirm the abnormality and determine whether they are increasing or decreasing. If an abnormality develops during the course of the study that meets any of the following criteria, the subject should be withdrawn:

- ALT or AST > 8x ULN;
- ALT or AST > 5x ULN for more than 2 weeks;
- ALT or AST > 3x ULN and (total bilirubin > 2x ULN or INR > 1.5); or
- ALT or AST > 3x ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (> 5%).

These subjects will also be closely monitored for symptoms of hepatitis, including acute viral hepatitis, alcoholic and autoimmune hepatitis, hepatobiliary disorders, nonalcoholic steatohepatitis, cardiovascular causes or concomitant treatments. Monitoring will also be performed for subjects whose serum transaminases double from their baseline value(s). If the repeat value for ALT or total bilirubin is unchanged or decreases, monitoring will continue at weekly intervals or until the results are acceptable or normalized. If any value has increased further, immediate close observation or discontinuation of drug will be followed.

Criteria for stopping the study or changing the study protocol

Criteria for stopping or changing the study protocol due to safety concerns are as follows:

- A blinded safety review of liver function tests will be conducted when 33%%, and 66% of the subjects have been enrolled. If 2 of the enrolled subjects in the study have persistent (e.g. confirmed on a repeated test and not resolved after 1 week) elevated liver function tests (AST, ALT) > 3xULN, the investigator or designee will convene an independent drug safety group to assess the safety profile of the investigational drug for this study. In addition, any hepatocellular injury meeting the definition of Hy's Law will require study enrollment to be halted until an independent drug safety group is convened and their recommendation is received. Any potential Hy's Law case will be handled as a suspected unexpected serious adverse reaction.
- Should the investigator, independent monitor, the FDA, or local regulatory authorities become aware of conditions arising during the conduct of this study that may warrant the cessation of the study, such action may be taken. Prior to such action, consultation between the investigator, and, as appropriate, the FDA and/or local regulatory authorities will take place
- If there are >2 subjects with serious adverse events who develop the same CTCAE Grade 3 criteria, the independent study monitor will examine and audit the cases and determine the relevance to the assigned study drug(s) and whether the trial should continue or if there are grounds for discontinuing the study.

10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitors, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Certificate of Confidentiality (CoC request to the NIH requires IRB approved protocol and ICF. This will be obtained after initial IRB approval.)

To further protect the privacy of study participants, a Certificate of Confidentiality will be issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative,

or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.1.4 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator	Study Monitors		
Bianca Chang, MD	Eric Vasiliauskas MD and Gil		
8,	Melmed MD		
Cedars-Sinai Medical Center	Cedars-Sinai Medical Center		
8700 Beverly Blvd	8700 Beverly Blvd		
(310) 423-0617	310-423-4100		
bianca.chang@cshs.org	Eric.Vasiliauskas@cshs.org;		
	Gil.Melmed@cshs.org		

10.1.5 SAFETY OVERSIGHT

Safety oversight will be under the direction of two independent Data and Safety Monitors with the appropriate expertise, including expertise in gastroenterology and IBS. The Data and Safety Monitors are independent from the study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflict of interest. The Data and Safety Monitors will assess safety and efficacy data on each arm of the study at least semiannually. A Data and Safety Monitoring Plan has been developed.

10.1.6 DATA HANDLING AND RECORD KEEPING

10.1.6.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into REDCap, a 21 CFR Part 11-compliant data capture system. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.1.6.2 STUDY RECORDS RETENTION

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonisation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

10.1.7 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents, reported to Data Safety Monitors. Protocol deviations must be sent to the reviewing Institutional Review Board (IRB) per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements.

10.1.8 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 ABBREVIATIONS

AE	Adverse Event			
CFR	Code of Federal Regulations			
COC	Certificate of Confidentiality			
CRF	Case Report Form			
EC	Ethics Committee			

eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GMP	Good Manufacturing Practices
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
IND	Investigational New Drug Application
IRB	Institutional Review Board
MOP	Manual of Procedures
NCT	National Clinical Trial
PI	Principal Investigator
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SOA	Schedule of Activities
SOP	Standard Operating Procedure
UP	Unanticipated Problem
US	United States

10.3 PROTOCOL AMENDMENT HISTORY

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale. A Summary of Changes table for the current amendment is located in the Protocol Title Page.

Version	Date	Description of Change	Brief Rationale

11 REFERENCES

- 1. Johansson ME, Sjovall H, Hansson GC. The gastrointestinal mucus system in health and disease. Nat Rev Gastroenterol Hepatol 2013;10:352-61.
- 2. Ermund A, Schutte A, Johansson ME, et al. Studies of mucus in mouse stomach, small intestine, and colon. I. Gastrointestinal mucus layers have different properties depending on location as well as over the Peyer's patches. Am J Physiol Gastrointest Liver Physiol 2013;305:G341-7.
- 3. Oriano M, Terranova L, Teri A, et al. Comparison of different conditions for DNA extraction in sputum a pilot study. Multidiscip Respir Med 2019;14:6.
- 4. Leite G, Morales W, Weitsman S, et al. Optimizing Microbiome Sequencing for Small Intestinal Aspirates: Validation of Novel Techniques through the REIMAGINE Study. BMC Microbiol 2019;19:doi:10.1186/s12866-019-1617-1.
- 5. Sadowska AM, Verbraecken J, Darquennes K, et al. Role of N-acetylcysteine in the management of COPD. Int J Chron Obstruct Pulmon Dis 2006;1:425-34.
- Mokhtari V, Afsharian P, Shahhoseini M, et al. A Review on Various Uses of N-Acetyl Cysteine. Cell J 2017;19:11-17.
- 7. Lembo A, Pimentel M, Rao SS, et al. Repeat Treatment With Rifaximin is Safe and Effective in Patients With Diarrhea-predominant Irritable Bowel Syndrome. Gastroenterology 2016;151:1113–1121.
- 8. Lovell RM, Ford AC. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. Clin Gastroenterol Hepatol 2012;10:712-721 e4.
- 9. Thompson WG. The functional gasterointestinal bowel disorders. In: Drossman DA, editor. The functional gaterointestinal disorders. Boston: Little, Brown; 1994. p. pp-117-134.
- 10. Neal KR, Barker L, Spiller RC. Prognosis in post-infective irritable bowel syndrome: a six year follow up study. Gut 2002;51:410-3.
- 11. The burden of gasterointestinal diseases. In: American Gastroenterological Association; 2001; Bethesda, MD; 2001.
- 12. Lackner JM, Gudleski GD, Zack MM, et al. Measuring health-related quality of life in patients with irritable bowel syndrome: can less be more? Psychosom Med 2006;68:312-20.
- 13. Okhuysen PC, Jiang ZD, Carlin L, et al. Post-diarrhea chronic intestinal symptoms and irritable bowel syndrome in North American travelers to Mexico. Am J Gastroenterol 2004;99:1774-8.
- 14. Mearin F, Perez-Oliveras M, Perello A, et al. Dyspepsia and irritable bowel syndrome after a Salmonella gastroenteritis outbreak: one-year follow-up cohort study. Gastroenterology 2005;129:98-104.
- 15. Ji S, Park H, Lee D, et al. Post-infectious irritable bowel syndrome in patients with Shigella infection. J Gastroenterol Hepatol 2005;20:381-6.
- 16. Spiller RC, Jenkins D, Thornley JP, et al. Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability following acute Campylobacter enteritis and in post-dysenteric irritable bowel syndrome. Gut 2000;47:804-11.
- 17. Pimentel M, Chow EJ, Lin HC. Eradication of small intestinal bacterial overgrowth reduces symptoms of irritable bowel syndrome. Am J Gastroenterol 2000;95:3503-6.

- 18. Pimentel M, Chow EJ, Lin HC. Normalization of lactulose breath testing correlates with symptom improvement in irritable bowel syndrome. a double-blind, randomized, placebo-controlled study. Am J Gastroenterol 2003;98:412-9.
- 19. Lupascu A, Gabrielli M, Lauritano EC, et al. Hydrogen glucose breath test to detect small intestinal bacterial overgrowth: a prevalence case-control study in irritable bowel syndrome. Aliment Pharmacol Ther 2005;22:1157-60.
- 20. Cuoco L, Salvagnini M. Small intestine bacterial overgrowth in irritable bowel syndrome: a retrospective study with rifaximin. Minerva Gastroenterol Dietol 2006;52:89-95.
- 21. Majewski M, McCallum RW. Results of small intestinal bacterial overgrowth testing in irritable bowel syndrome patients: clinical profiles and effects of antibiotic trial. Adv Med Sci 2007;52:139-42.
- 22. Shah ED, Basseri RJ, Chong K, et al. Abnormal breath testing in IBS: a meta-analysis. Dig Dis Sci 2010;55:2441-9.
- 23. Posserud I, Stotzer PO, Bjornsson ES, et al. Small intestinal bacterial overgrowth in patients with irritable bowel syndrome. Gut 2007;56:802-8.
- 24. Pyleris E, Giamarellos-Bourboulis EJ, Tzivras D, et al. The prevalence of overgrowth by aerobic bacteria in the small intestine by small bowel culture: relationship with irritable bowel syndrome. Dig Dis Sci 2012;57:1321-9.
- 25. Giamarellos-Bourboulis EJ, Pyleris E, Barbatzas C, et al. Small intestinal bacterial overgrowth is associated with irritable bowel syndrome and is independent of proton pump inhibitor usage. BMC Gastroenterol 2016;16:67.
- 26. Pimentel M, Morales W, Pokkunuri V, et al. Autoimmunity Links Vinculin to the Pathophysiology of Chronic Functional Bowel Changes Following Campylobacter jejuni Infection in a Rat Model. Dig Dis Sci 2015;60:1195-205.
- 27. Pimentel M, Morales W, Rezaie A, et al. Development and validation of a biomarker for diarrheapredominant irritable bowel syndrome in human subjects. PLoS One 2015;10:e0126438.
- 28. Shenker BJ, Hoffmaster RH, Zekavat A, et al. Induction of apoptosis in human T cells by Actinobacillus actinomycetemcomitans cytolethal distending toxin is a consequence of G2 arrest of the cell cycle. J Immunol 2001;167:435-41.
- 29. Purdy D, Buswell CM, Hodgson AE, et al. Characterisation of cytolethal distending toxin (CDT) mutants of Campylobacter jejuni. J Med Microbiol 2000;49:473-9.
- 30. Pickett CL, Whitehouse CA. The cytolethal distending toxin family. Trends Microbiol 1999;7:292-7.
- 31. Eshraghi A, Maldonado-Arocho FJ, Gargi A, et al. Cytolethal distending toxin family members are differentially affected by alterations in host glycans and membrane cholesterol. J Biol Chem;285:18199-207.
- 32. Smith JL, Bayles DO. The contribution of cytolethal distending toxin to bacterial pathogenesis. Crit Rev Microbiol 2006;32:227-48.
- 33. Frisk A, Lebens M, Johansson C, et al. The role of different protein components from the Haemophilus ducreyi cytolethal distending toxin in the generation of cell toxicity. Microb Pathog 2001;30:313-24.
- 34. Torihashi S, Ward SM, Nishikawa S, et al. c-kit-dependent development of interstitial cells and electrical activity in the murine gastrointestinal tract. Cell Tissue Res 1995;280:97-111.

- 35. Rumessen JJ. Ultrastructure of interstitial cells of Cajal at the colonic submuscular border in patients with ulcerative colitis. Gastroenterology 1996;111:1447-55.
- 36. Streutker CJ, Huizinga JD, Campbell F, et al. Loss of CD117 (c-kit)- and CD34-positive ICC and associated CD34-positive fibroblasts defines a subpopulation of chronic intestinal pseudo-obstruction. Am J Surg Pathol 2003;27:228-35.
- 37. Vanderwinden JM, Liu H, De Laet MH, et al. Study of the interstitial cells of Cajal in infantile hypertrophic pyloric stenosis. Gastroenterology 1996;111:279-88.
- 38. Ordog T, Takayama I, Cheung WK, et al. Remodeling of networks of interstitial cells of Cajal in a murine model of diabetic gastroparesis. Diabetes 2000;49:1731-9.
- 39. Bassotti G, Villanacci V, Maurer CA, et al. The role of glial cells and apoptosis of enteric neurones in the neuropathology of intractable slow transit constipation. Gut 2006;55:41-6.
- 40. Pimentel M, Chatterjee S, Chow EJ, et al. Neomycin improves constipation-predominant irritable bowel syndrome in a fashion that is dependent on the presence of methane gas: subanalysis of a double-blind randomized controlled study. Dig Dis Sci 2006;51:1297-301.
- 41. Sharara AI, Aoun E, Abdul-Baki H, et al. A randomized double-blind placebo-controlled trial of rifaximin in patients with abdominal bloating and flatulence. Am J Gastroenterol 2006;101:326-33.
- 42. Pimentel M PS, Kane, SV, Kong Y. Rifaximin, a non-absorbable antibiotic improves the symptoms of irritable bowel syndrome: A randomized, double-blind, placebo-controlled study. Ann Intern Med 2006;145:557-563.
- 43. Pimentel M, Lembo A, Chey WD, et al. Rifaximin therapy for patients with irritable bowel syndrome without constipation. N Engl J Med 2011;364:22-32.
- 44. FDA approves two therapies to treat IBS-D. In: U.S. Food and Drug Administration; 2015.
- 45. Debbia EA, Maioli E, Roveta S, et al. Effects of rifaximin on bacterial virulence mechanisms at supra- and sub-inhibitory concentrations. J Chemother 2008;20:186-94.
- 46. Jiang ZD, DuPont HL. Rifaximin: in vitro and in vivo antibacterial activity--a review. Chemotherapy 2005;51 Suppl 1:67-72.
- 47. Gerard L, Garey KW, DuPont HL. Rifaximin: a nonabsorbable rifamycin antibiotic for use in nonsystemic gastrointestinal infections. Expert Rev Anti Infect Ther 2005;3:201-11.
- 48. FDA. FDA approves two therapies to treat IBS-D [news release]. In. Silver Spring, MD; 2015.
- 49. Fodor A, Pimentel M, Chey WD, et al. Rifaximin is associated with modest, transient decreases in multiple taxa in the gut microbiota of patients with diarrhoea-predominant irritable bowel syndrome. Gut Microbes 2018.
- 50. Pimentel M, Lembo A. Microbiome and Its Role in Irritable Bowel Syndrome. Dig. Dis. Sci. 2020;65:829-839.
- 51. Lacy BE, Mearin F, Chang L, et al. Bowel Disorders. Gastroenterology 2016;150:1393-1407.e5.